

On micro-bead mechanics with actin filaments

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Experiments have been performed using microscopic beads to probe the small scale mechanics of actin solutions. We show that there are a number of regimes possible as a function of the size of the probing particle. In certain cases we argue that the quasi-static response resembles a smectic crystal rather than an isotropic solid, implying an anomalous scaling of the mechanical response of actin solutions as a function of the size of the probing particles.

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The mechanics and rheology of actin filaments are a beautiful model system for the study of the dynamics and mechanics of semi-dilute polymers [1,2]. They are characterized by length scales which are easily accessible with optical techniques allowing the detailed study of phenomena such as tube dynamics. However, the macroscopic rheology of these systems has been hard to master from the experimental point of view. Difficulties of purification and sample preparation lead to orders of magnitude variations in such fundamental objects such as the value of the plateau modulus [3–7], the standard measure of the response of an entangled polymer solution to external perturbations.

To get around these problems of macroscopic sample preparation and also to probe the local viscoelastic behaviour of these materials a number of experimental groups have started using small, colloidal beads to study the local mechanics of these materials [8–13]. One either pulls on the particles using super-paramagnetic beads in a magnetic field, or one simply observes the fluctuations of the particles undergoing Brownian motion. In this letter I shall try to attack the problem as to what exactly one measures in these experiments. In particular how large do these particles have to be in order to measure a macroscopic elastic modulus and when do we expect to be sensitive to the individual filament properties?

In contrast with flexible polymers solutions there are two principal length scales present in a semi-dilute solution of actin: the mesh size and the persistence length. Naive application of scaling ideas thus becomes a highly ambiguous exercise because an arbitrarily large number of intermediated lengths can be created by considering $\xi^{1-\alpha}l_p^\alpha$ with ξ the mesh size and l_p the persistence length. This ambiguity in lengths also translates into an ambiguity in the plateau modulus which can be expressed as $k_B T$ per characteristic volume. As an example of this difficulty we might quote two attempts to calculate

the modulus in actin solutions with scaling approaches [14,15] where two completely different results are found due in part due to this problem. Indeed this proliferation of lengths is already known for the tube geometry where one finds both $\alpha = +1/5$ and $\alpha = -1/5$, [16–18]. We shall show in this article that a new intermediate scale with $\alpha = 3/5$ becomes crucial in the understanding of the elasticity of actin solutions at length scales probed with micrometer sized beads. At these scales we show that the elastic response is highly anisotropic and resembles that of a smectic, with anomalous penetration of the response into the sample and unusual scaling of the response with the size of the probing particle.

Note that in this letter I am interested in the low frequency mechanics and thus I exclude from the discussion high frequency fluctuation measurements (up to 20KHz) which have been recently performed [12] and am interested in a quasi-static regime between 10^{-3}Hz and 10Hz . The reason for considering this range scale will become clear during the discussion.

A coherent picture of the large scale mechanics of non-crosslinked actin solutions is now available. The actin system is usually polymerized [11] in conditions such that the mean distance between filaments, ξ is between 0.3μ and 1μ . ξ can be linked with the concentration of monomers c by noting that $\xi \sim 1/\sqrt{cd}$ with d the size of actin monomers. A useful geometric quantity is the length of filament per unit volume $\rho \sim 1/\xi^2$. The filament is characterized by its persistence length l_p which is close to 15μ [19]. For a single weakly bent filaments the energy of a configuration is given by [20]

$$E = k_B T l_p / 2 \int (\partial_s^2 \mathbf{r}_\perp(s))^2 ds \quad (1)$$

where $\mathbf{r}_\perp(s)$ is the transverse fluctuation of the filament about its equilibrium shape.

In a manner which is familiar from flexible polymers the individual filaments are confined to a tube whose diameter scales as $\xi^{6/5}/l_p^{1/5}$ and the filament is confined to the tube by collisions between the filament and its neighbors every $l_e \sim \xi^{4/5}l_p^{1/5}$ [16,17]. l_e is in some ways equivalent to the entanglement length in the Doi-Edwards tube model [21–23]. The long time dynamics and mechanics are dominated by the reptation of filaments along their tubes [5,23,25]. This process has a characteristic time, the reptation time, which defines the time scale beyond which the sample behaves like a viscous fluid (rather than an elastic solid) and can be as long

as several hours [5]. Under macroscopic shear the longitudinal stresses in a filament relax relatively rapidly [25] leaving a residual contribution to the free energy which comes from the modification of the free energy of confinement of the filament in its tube. A simple argument for this free energy is to count $k_B T$ per collision of the tube with the filament. Thus the macroscopic modulus varies as $G \sim \rho k_B T / l_e \sim c^{1.4} / l_p^{1/5}$ as confirmed by an explicit calculation [23,24].

This picture of filaments confined to a tube is only true on time scales that are long enough for the filament to dynamically sample fluctuations on the scale of l_e . This time, which is determined by the bending elasticity of the filaments varies as $\tau_e \sim \eta l_e^4 / l_p k_B T \sim 10 Hz$ [24]. This is our reason for restricting our treatment to lower frequencies, at higher frequencies one is presumably sensitive to individual filament dynamics (coupled by hydrodynamics) rather than the collective, entangled, modes that interest us in this letter. For frequencies lower than the inverse reptation time (ie frequencies comparable to $10^{-3} Hz$) the sample behaves as a fluid and the bead moves freely as filaments slide out of the way of the particles.

Before passing to the problem of the behavior of actin solutions we shall revise a Peierls like argument from which we can deduce the basic scaling behaviour of a normal elastic solid. We shall then adapt this argument to the case of semiflexible filaments: Consider a bead of radius R embedded in an elastic medium in d dimensions. If we pull on the particle with a force f we can make the following variational ansatz in order to find the minimum energy configuration. Let us assume that the material is disturbed over a distance l from the bead then the elastic energy, will scale in the following manner

$$E_{var} \sim G \int (\nabla a)^2 dV \quad (2)$$

where a is an amplitude of displacement, G an elastic constant and the integral is over the variational volume $V \sim l^d$. This scales as

$$E_{var} \sim G(a/l)^2 l^d \quad (3)$$

We see that in less than two dimension an arbitrarily small force is able to displace the bead large distances because E_{var} can be made small by increasing the variational parameter l . In three dimensions, however, the energy diverges with l and has a lower bound for small l due to the short wavelength cutoff coming from the finite size of the bead. Thus the minimum energy is found for $l \sim R$ and we deduce that $E_{var} \approx G a^2 R$. At constant force the displacement scales inversely with the bead size,

$$a \approx f / GR \quad (4)$$

A full calculation of the response of an isotropic viscoelastic material has recently been performed and confirms this simple scaling argument [12].

We see that there is an anisotropy in the problem coming from the direction in which we apply the force f , and we should worry that the volume excited is not spherical as has been assumed in the argument. Let us perform a slightly more elaborate variational treatment where we assume that the volume V is characterized by a disk of dimensions $l \times l \times D$ where the particle excites modes of wavelength l which penetrate D into the sample in the direction of f . In this case our estimate for E_{var} is

$$E_{var} \sim (l^2 D) G ((a/l)^2 + (a/D)^2) \quad (5)$$

Where a/l and a/D are the estimates of the components of the strain tensor in the material. Taking D as a variational parameter one sees that $D \sim l$ and the problem reduces to that considered above. This is in fact a crude statement of the principle of St. Vernet that a force on a body with a wavelength l decays into the body over the same length scale, which is a elementary property of periodic harmonic functions in three dimensions.

How must this argument be modified in the actin system? Experiments are performed with bead which vary in size from $.1\mu$ and 10μ . The smallest beads pass between the filaments and diffuse almost freely [11]; they will not concern us any further. Are we able to use continuum elastic arguments (like that above) to deduce the experimental stress strain relationships? We now argue that in actin solutions there are now two contributions to the variational energy E_{var} . For large beads the normal continuum elasticity (summarized above) dominates, for smaller beads however a new, and novel elastic response is found: Consider a volume V distorted by a force on a particle of size R . Again we take this volume as anisotropic with dimensions $l \times l \times D$. In this volume the filaments which traverse the volume bend with a wavelength l and there is a bending contribution to the total energy, coming from eq. (1) which varies as

$$E_1 \sim (l^2 D) (a^2 k_B T l_p / l^4) \rho \quad (6)$$

The three multiplicative factors are respectively the volume excited, the bending energy per unit length of filament and the filament density within the volume. a is again the typical amplitude of the excitation in the volume. To this bending contribution one must add the equivalent of E_{var} . When we impose the bending on the volume V there is also a variation in the geometry of the confining tubes. For instance in the direction of f the tubes are compressed by a factor comparable to a/D . Thus there is thus a contribution to the energy coming from the macroscopic bulk modulus of the form

$$E_2 \sim (l^2 D) ((a/l)^2 + (a/D)^2) (\rho k_B T / l_e) \quad (7)$$

where we have again respectively the volume, the square elastic stress and the macroscopic elastic modulus. We can now optimize $E_1 + E_2$ by minimizing over D . However, we first notice that there are two term linear in D

and that depending on the value of l one or the other will dominate. If we look at wavelengths

$$l < l_c = \sqrt{l_e l_p} \sim \xi^{2/5} l_p^{3/5} \quad (8)$$

the contribution from E_1 dominates over that from E_2 . When $l > l_c$ the second contribution dominates.

We conclude that there is an important new length scale in the problem. When we look at excitations with wavelengths greater than l_c the two contributions in E_2 are going to dominate the elasticity and we are back to the case of normal continuum elastic theory. However in the short wavelength limit $l < \sqrt{l_e l_p}$ we find that the elastic energy is given by

$$E_{eff} \sim a^2 \rho (l^2 D) (k_B T / D^2 l_e + l_p / l^4) \quad (9)$$

Minimizing the energy over D we find several surprising results. Firstly

$$l^2 \sim D \sqrt{l_e l_p} \quad (10)$$

the theorem of St. Vernet does not apply. Secondly substituting eq. (10) in (9) gives

$$E_{eff} \sim \rho a^2 k_B T \sqrt{\frac{l_p}{l_e}} \quad (11)$$

which should be compared with the corresponding result for a normal solid eq. (3). From eq. (10) we see that the volume excited scales in an anisotropic fashion with the wavelength, quite unlike normal elastic solids. One is reminded of the penetration of excitation into a smectic liquid crystal with an effective energy for fluctuations of the form $E \sim K \int [(\partial_x^2 u)^2 + (\partial_y^2 u)^2 + \beta (\partial_z u)^2] dV$ where the z axis is defined by the direction of application of the force.

The length scale l is absent in the energy (11) which has not been minimized over the wavelength; this is analogous to the case of a normal elastic material eq. (3) in two rather than three dimensions. It suggests that we are in the lower critical dimension for the problem (at least in a certain range of wavelengths) and that a fuller treatment will bring out logarithmic corrections to our picture. We also deduce, since eq. (11) is independent of l , that the amplitude of displacement of a particle should be independent of its size, in contrast to the dependence discussed above eq. (4): We are no longer dominated by the short wavelength cutoff in the energy integral eq. (2) despite being in three dimensions

Finally we note that for coherency in this picture we require that the depth of penetration of the excitation into the sample D be greater than the distance between the filaments ξ , otherwise a continuum description as used here must break down. This implies that $l > l_l = \sqrt{\xi \sqrt{l_e l_p}} = \xi^{7/10} l_p^{3/10}$. On wavelengths shorter

than this, one is presumably sensitive to filaments directly in contact with the probing particle and do not feel the three dimensional nature of the sample.

Substituting typical values for material constants, $\xi \sim .5\mu$, $l_p \sim 15\mu$ we find that $l_e \sim 1\mu$. The crossover length scale $l_c \sim 4\mu$. The short wavelength cutoff $l_l \sim 1.5\mu$. We thus expect the following series of crossovers as a function of probing wavelength. (a) For $\xi < l < l_l$ one probes the bending of individual filaments. (b) For $l_l < l < l_c$ collective excitations of the solution become important with anomalous penetration of the excitation into the sample. (c) For $l > l_c$ the elasticity becomes isotropic. These crossovers are too closely spaced to be experimentally studied in great detail, however we conclude that to measure a valid macroscopic response function particle sizes should be substantially greater than $l_c = 4\mu$.

Until now we have only considered the low frequency response of a sample, that is for times long enough that all longitudinal stresses have relaxed along the tube. It has been shown [25] that one expects two plateau moduli as a function of frequency. The low frequency plateau used in the above discussion comes from variation in tube geometry under sample shear. The second much larger contribution which dominates at higher frequencies comes from coupling of the shear to the longitudinal density fluctuations of the filament in its tube. Can we see the crossover between the low frequency and high frequency behaviour with micro-bead techniques? This question is difficult to answer, the static approach used above is not adapted to answer this dynamic question however we can certainly expect that the frequency of crossover between the two regimes will vary with the bead size.

The regime of the high plateau in macroscopic rheology is delimited by the two times $\tau_e \sim 0.1s$ and $\tau_e (l_p / l_e)^2 \sim 10s$. This second time is the time needed for excitations to diffuse a distance l_p along the tube. It is important because macroscopic shear produces density fluctuations along the tube which are coherent over a distance l_p . When we excite a sample with a wavelength l , which is smaller the l_p , we expect that the window of times for the observation of this high plateau is reduced to the interval between τ_e and $\tau_e (l / l_e)^2$. For the smallest beads this high second plateau should almost completely disappear. Even with larger beads the elastic modulus should be substantially underestimated over certain frequency ranges. More detailed discussion of this regime seems to be difficult without a detailed *dynamic* theory of the coupling of the bend and longitudinal degrees of freedom.

To conclude actin mechanics shows a quite rich series of crossover in the response function $G(q, \omega)$. We have simple arguments for the wavevector dependence of this function at frequencies between $10^{-3}Hz$ and $10Hz$. Further work requires a full dynamic theory of the coupling between bending and density fluctuations. $G(q, \omega)$

is more complicated than might be expected; recent experiments which interpret elastic stress propagation in terms of an isotropic elastic theory characterized by an elastic constant and Poisson ratio [26] may be missing some interesting physics on length scales smaller than 4μ .

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- [1] J. Kas, H. Strey, E. Sackmann, *Nature* **368**, 226 (1994).
 - [2] J. Kas, H. Strey J.X. Tang, D. Finger, R.Ezzell, E. Sackmann P. Janmey **70** 609 (1995).
 - [3] T.D. Pollard, I. Goldberg W.H. Schwarz *J. Biol Chem* **267** 20339 (1992).
 - [4] K.S. Zaner *J. Biol Chem* **261** 7615 (1986).
 - [5] O. Muller, H. E. Gaub, M. Barmann, E. Sackmann, *Macromolecules* **24**, 3111 (1991).
 - [6] R. Ruddies W.H. Goldmann, G. Isenberg E. Sackmann *Eur. Biophys J* **22** 309 (1993).
 - [7] P. Janmey, S. Hvidt, J. Peetermans, J. Lamb, JD Ferry, TP Stossel *Biochem* **27** 8218 (1988).
 - [8] K.S. Zaner P.A. Valberg *J. Cell Bio.* **109** 2233 (1989).
 - [9] F. Ziemann, J. Radler, E. Sackmann, *Biophysical J.* **66**, 1 (1994).
 - [10] F. Amblard, A. C. Maggs , B. Yurke, A. Pargellis and S. Leibler *Phys Rev Lett.* **77** 4470 (1996).
 - [11] C. F. Schmidt, M. Brmann, G. Isenberg, E. Sackmann, *Macromolecules* **22**, 3638 (1989).
 - [12] B. Schnuee, F. Gittes, P.D. Olmsted C.F. Schmidt F.C. MacKintosh Preprint. available at <http://www-personal.umich.edu/~fcm/>
 - [13] D. Weitz MRS abstract Dec 1996, A. Palmer, K Rufner, D. Wirtz Preprint.
 - [14] F. Mackintosh P. Janmey J. Kas, *Phys Rev Lett.* **75** 4425 (1995).
 - [15] K. Kroy, E. Frey. *Phys Rev Lett* **77** 306 (1996)
 - [16] A.N. Semenov, *J. Chem Soc. Faraday Trans 2*, **82** 317 (1986).
 - [17] A.N. Semenov *Physica* **A166** 263 (1990).
 - [18] T. Odijk *Macromolecules* **19** 2073 (1986).
 - [19] F. Gittes, B. Mickey, J. Nettleton, J. Howard, *J. Cell Biol.* **120**, 923 (1993);
 - [20] L. Landau, E. M. Lifshitz, *Theory of Elasticity* (Pergamon Press, Oxford) 1986.
 - [21] M. Doi and S.F. Edwards *Dynamics of polymer solutions* (Oxford University press, 1986)
 - [22] P.G. de Gennes *Scaling theory of polymer physics* (Cornell University press, Ithaca, 1979).
 - [23] D. Morse Preprint, University of Minnesota (1997).
 - [24] H. Isambert, A.C. Maggs, *Macromolecules* **29** 1036 (1996).
 - [25] A. C. Maggs, *Phys. Rev. E.* **55** June (1997). Available as cond-mat/9704126
 - [26] F.G. Schmidt F. Ziemann, E.Sackmann *Eur Biophys J* **24** 348 (1996)